

## **Systematic Investigation of the Effects of Multiple SV40 Nuclear Localization Signal Fusion on the Genome Editing Activity of Purified SpCas9**

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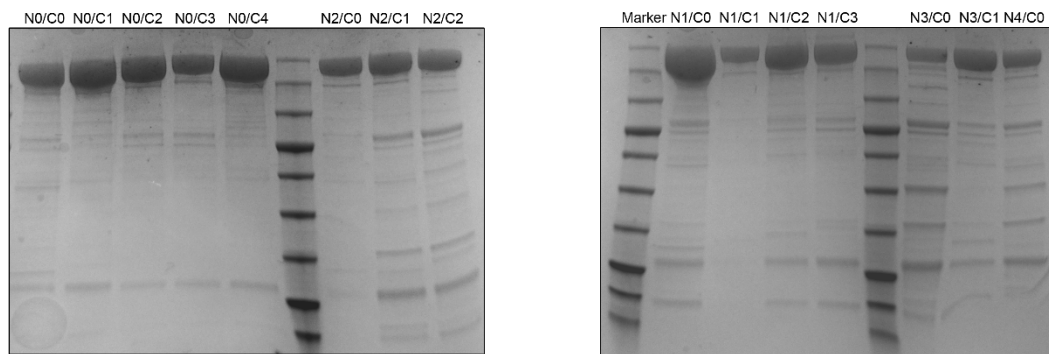
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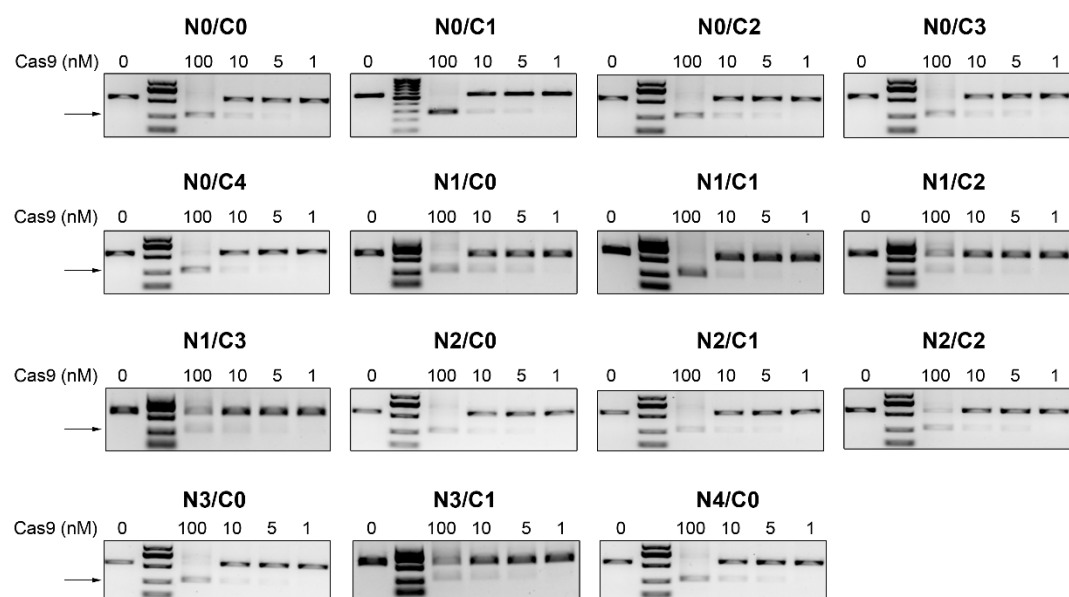
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Switzerland; Swiss Institute of Bioinformatics (SIB), Lausanne CH-1015, Switzerland

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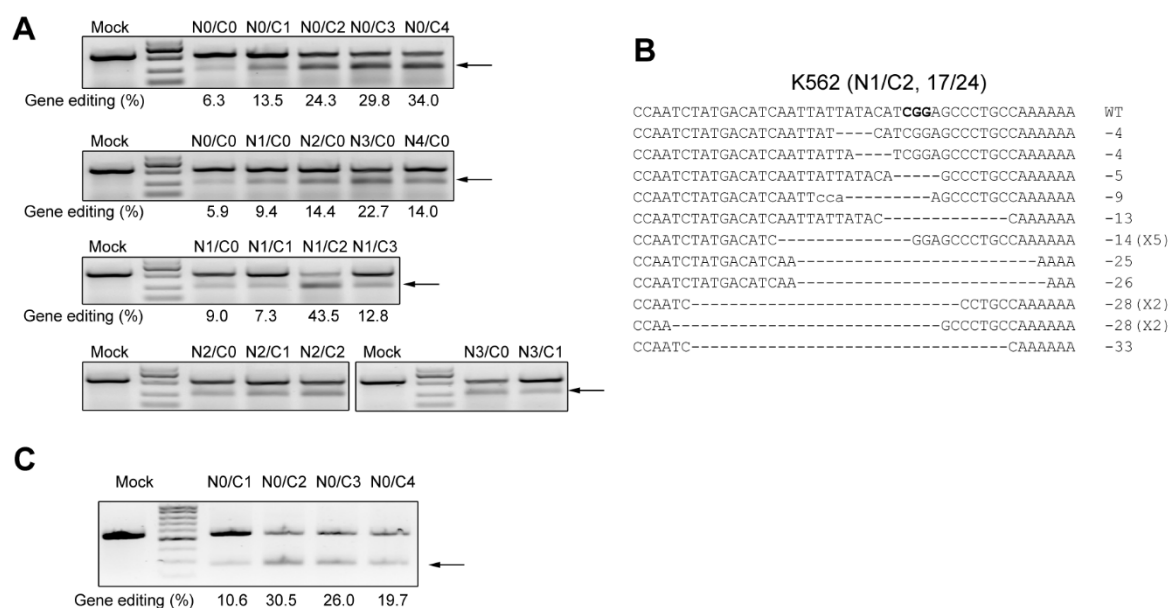
## Supplemented Figures



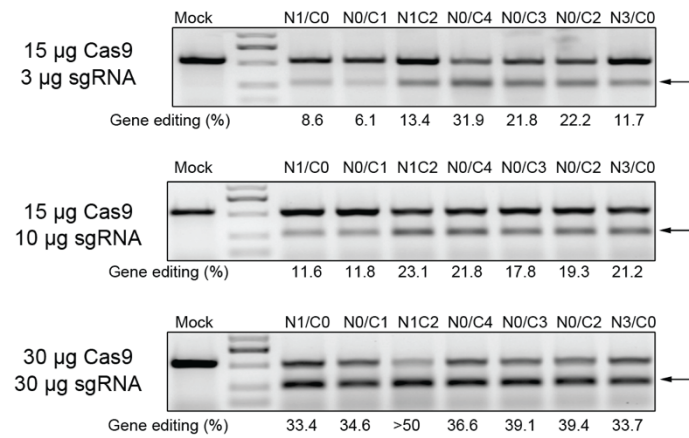
**Figure S1. SDS-PAGE of purified SpCas9 proteins in this study.**



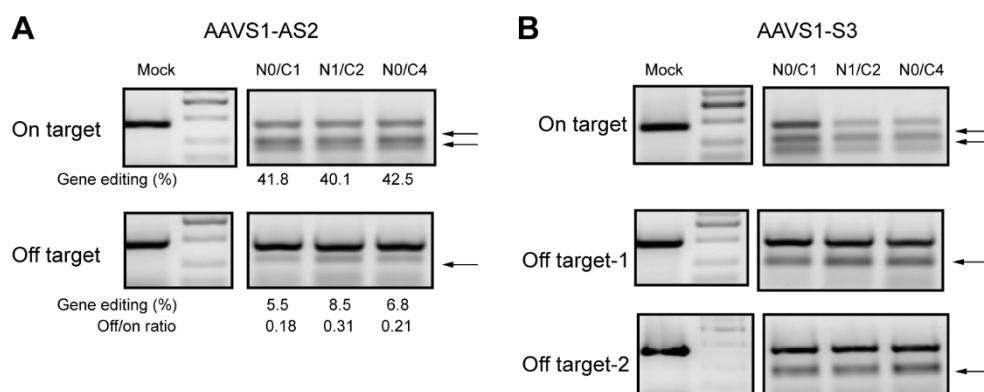
**Figure S2. Evaluation of the *in vitro* activity of multi-NLS SpCas9 variants, determined by 1% agarose gel electrophoresis.** Cleaved substrate DNA are indicated by arrows.



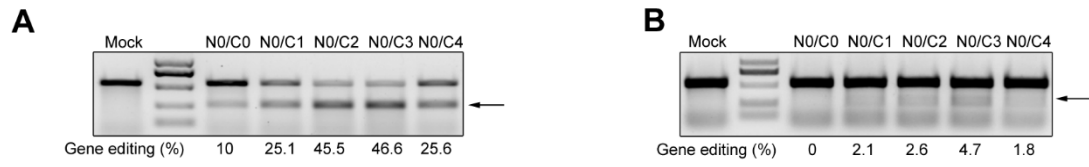
**Figure S3. Evaluation of the genome-editing activity of nucleofected multi-NLS SpCas9 proteins in K562 (A-B) and Jurkat (C) cells.** (A) Representative images of T7E1 analysis of the genome-editing activity of SpCas9 variants in K562 (Related to Figure 3A). The cleavage product is indicated by arrows. (B) Validation of the modified genomic locus by Sanger sequencing (Related to Figure 3A). (C) Representative images of T7E1 analysis of the genome-editing activity of SpCas9 variants in Jurkat (Related to Figure 3B). The cleavage product is indicated by arrows. T7E1 assay is performed with 1% agarose gel electrophoresis unless noted otherwise.



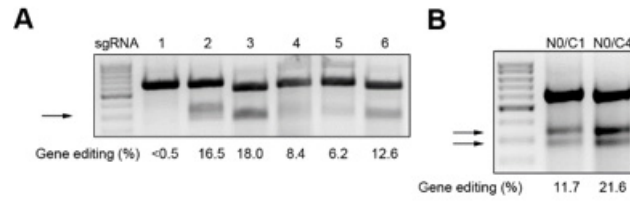
**Figure S4 (related to Figure 4A). The effects of multi-NLS on SpCas9 proteins at different dosage, determined by T7E1 assay on 1% agarose gel.**



**Figure S5 (related to Figure 4B). The effects of multi-NLS on the specificity of SpCas9 variants, determined by T7E1 assay on 1% agarose gel. (A) On-target and off-target activities of sgRNA AAVS1-AS2. (B) On-target and off-target activities of sgRNA AAVS1-S3. The arrows indicate cleavage products.**



**Figure S6 (related to Figure 5). Representative images of the T7E1 results of gene-modified hNPCs (A) and hDFs (B) with multi-NLS SpCas9 proteins.** Arrows indicate cleavage product. T7E1 results are resolved on 1% agarose gel.

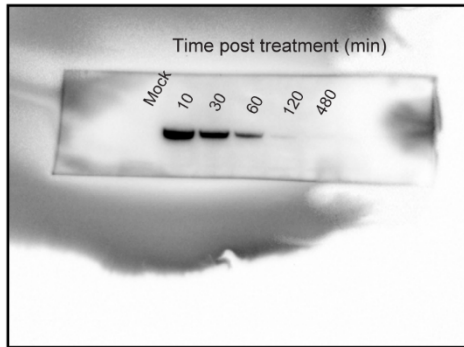


**Figure S7 (related to Figure 6). Genome editing of mouse embryos using microinjected N0/C1 and N0/C4 SpCas9 proteins.** (A) T7E1 analyses of PTEN-targeting sgRNA screening in mouse Hepa 1-6 cell line. (B) T7E1 analyses of pooled 2-cell embryos treated with N0/C1 and N0/C4 SpCas9 proteins respectively. Arrows indicate cleavage product. T7E1 results are resolved on 1% agarose gel.

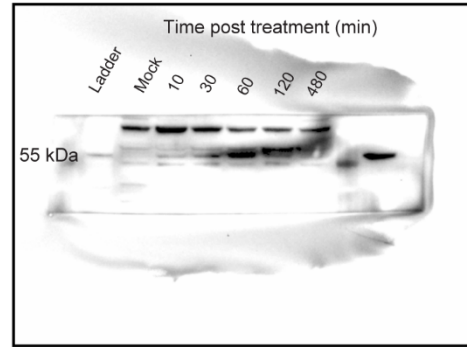
## Uncropped western blot (related to Figure 3C-D)

### Figure 3C

HA



Lamin B1

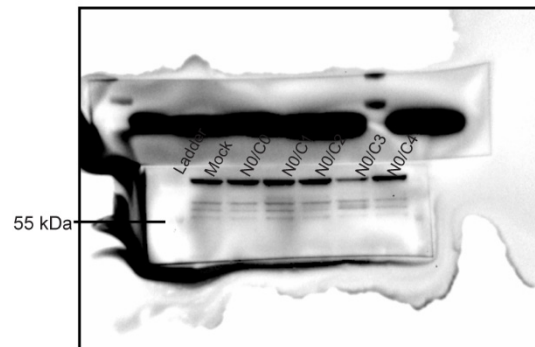


### Figure 3D

HA



Lamin B1



## Supplementary Tables

**Table S1. sgRNA used in this study**

<b>Name</b>	<b>Target gene</b>	<b>Sequence</b>
CCR5-sgRNA	CCR5	TGACATCAATTATTATACAT
293-Reporter-sgRNA-1	EGFP	CAGAATTGATACTGACTGTA
293-Reporter-sgRNA-2	EGFP	GATTCAGTCAGTATCAATTC
AAVS1-AS2	AAVS1	CTCCCTCCCAGGATCCTCTC
AAVS1-S3	AAVS1	GGGAGGGAGAGCTTGGCAGG
EMX1-sgRNA	EMX1	GTCACCTCCAATGACTAGGG
mouse-pTen-sgRNA-1	PTEN	AATATTATTGCTATGGGATT
mouse-pTen-sgRNA-2	PTEN	ACAATATTGATGATGTAGTA
mouse-pTen-sgRNA-3	PTEN	ACCGCCAAATTTAACTGCAG
mouse-pTen-sgRNA-4	PTEN	GCAGCAATTCACTGTAAAGC
mouse-pTen-sgRNA-5	PTEN	TGTCATCTTCACTTAGCCAT
mouse-pTen-sgRNA-6	PTEN	CATACCTCTGCAGTTAAATT

**Table S2. Primers used in this study**

Name	Sequence
CCR5-EX-F	AGGTGAGAGGATTGCTTG
CCR5-EX-R	AAATGAGAGCTGCAGGTG
CCR5-IN-F	GAGCCAAGCTCTCCATCTAGT
CCR5-IN-R	GCCCTGTCAAGAGTTGACAC
AAVS1-EX-F	AGCCTGAGCGCCTCTCCT
AAVS1-EX-R	TTGCTTTCTTTGCCTGGA
AAVS1-IN-F	TGCTTCTCCTCTTGCGAAGT
AAVS1-IN-R	CGGTTAATGTGGCTCTGGTT
EMX1-EX-F	AGAGGAGCTAGGATGCAC
EMX1-EX-R	TGAATTACCCTTGACCCC
EMX1-IN-F	GGAGCAGCTGGTCAGAGGGG
EMX1-IN-R	AATCTACCACCCCAGGCTCT
AAVS1-AS2-OT-EX-F	CAGGCTCCTTGTTCTTCTGG
AAVS1-AS2-OT-EX-R	AAGCCATGGTCTGCTTCACT
AAVS1-AS2-OT-IN-F	TGCAGGAAAAAGTTGCAGTG
AAVS1-AS2-OT-IN-R	GGCCAAACTGGAGAGACAGA
AAVS1-S3-OT1-EX-F	CAGCTTCCTGGAAAGACCTG
AAVS1-S3-OT1-EX-R	GGTTTTGGCCTGACTGTGTT
AAVS1-S3-OT1-IN-F	TATCTCCTCTCCCCCTGCTT
AAVS1-S3-OT1-IN-R	GAGCTGCCTGACTTCCCTAA
AAVS1-S3-OT2-EX-F	AGCTTCTGCAACTGGTGGTT
AAVS1-S3-OT2-EX-R	AGTTGCTGGTAGGAGGCTGA
AAVS1-S3-OT2-IN-F	GGATGAGAGAGCTGGTTTGC
AAVS1-S3-OT2-IN-R	AGGCTTCTCTTCCCTCGTTC
EMX1-EX-F	TTGAGATGGCTGTTCAAGAG
EMX1-EX-R	TTGAGACATGGGGATAGAATCA
EMX1-IN-F	GTGGGGAGATTTGCATCTGT
EMX1-IN-R	CAATGGGAAGGACAGCTTCT
pTen-1/2-EX-F	CCAGAATATGTAGGCGGAGG
pTen-1/2-EX-R	GTGGGCCTTGAGAAACACTT
pTen-1/2-IN-F	GAGCCTCAGTCGCGTATTCT
pTen-1/2-IN-R	CAAGCCAGAGAATAACCTGG
pTen-3/6-EX-F	AACATGTATGTTTTAAGTAA
pTen-3/6-EX-R	TGTACATTTAAAGCTAAGTG
pTen-3/6-IN-F	GCATGCATGCGTTTAACTTT
pTen-3/6-IN-R	AGCGCTAGCTGTCCCACACC
pTen-4/5-EX-F	CAAGTCACTGGGTAAATTTA
pTen-4/5-EX-R	CATGCCACTAATTCAACAGT
pTen-4/5-IN-F	CCATGTCTAATGAATGCATT
pTen-4/5-IN-R	CAGTTCTCAAAGCATCACAC

Mycoplasma test-F	GGCGAATGGGTGAGTAACACG
Mycoplasma test-R	CGGATAACGCTTGCGACCTATG

## Supplementary Methods

### 1. Sequences of Cas9 proteins

#### SpyCas9 core sequence

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKR  
TARRRYTRRKNRICYLQEIFSNEMAKVDDSSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYP  
TIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPI  
NASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNFKSNFDAEDAKLQLSK  
DTYDDDDLNDLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLL  
KALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRK  
QRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYYVGPLARGNSRFAWMTRKS  
EETITPWNFEFVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR  
KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK  
DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLIN  
GIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAICK  
GILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIIEGKELGSQILKEHPV  
ENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVHDIVPQSFLKDDSIDNKVLTRSDKNRGK  
SDNVPSEEVVKMKKNYWRQLLNAKLITQRKFDNLTAKAERGGLSELDKAGFIKRQLVETRQITKHV  
AQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTA  
LIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE  
TNGETGEIVWDKGRDFATVRKVLSPQVNVIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK  
KYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLII  
KLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKKGSPEDNEQKQLFVEQ  
HKHYLDEIIEQISEFSKRVLADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDT  
TIDRKRYTSTKEVLDTLIHQSIITGLYETRIDLSQLGGD

#### NLS Cas9 variants

(color shown as magenta for HA tag, green for His<sub>6</sub> tag and yellow for NLS sequence)

#### N0/C0 SpyCas9

[SpyCas9 Core Sequence]-GGSYPYDVPDYALEGGGSHHHHHH

#### N0/C1 SpyCas9

[SpyCas9 Core Sequence]-GGSYPYDVPDYA  
LEGGGRGGSPPKKRKYVGGSHHHHHH

#### N0/C2 SpyCas9

[SpyCas9 Core Sequence]-GGSYPYDVPDYALEGGGSPPKKRKYVGGRGGSPPKKRKYVGGSHHHHHH

### N0/C3 SpyCas9

[SpyCas9 Core Sequence]-

GGSSYPYDVDPDYALEGGGSPKKKRKVGGSPKKKRKVGGRGGSPPKKKRKVGGSHHHHHH

### N0/C4 SpyCas9

[SpyCas9 Core Sequence]-

GGSSYPYDVDPDYALEGGGSPKKKRKVGGSPKKKRKVGGSPKKKRKVGGRGGSPPKKKRKVGGSHHHHH

### N1/C0 SpyCas9

MGSSPPKKKRKVGGSTS-[SpyCas9 Core Sequence]-GGSSYPYDVDPDYALEGGGSHHHHHH

### N1/C1 SpyCas9 (Yellow, NLS; magenta, HA; green, His<sub>6</sub> tag)

MGSSPPKKKRKVGGSTS-[SpyCas9 Core Sequence]-

GGSSYPYDVDPDYALEGGGRGGSPPKKKRKVGGSHHHHHH

### N1/C2 SpyCas9

MGSSPPKKKRKVGGSTS-[SpyCas9 Core Sequence]-

GGSSYPYDVDPDYALEGGGSPKKKRKVGGRGGSPPKKKRKVGGSHHHHHH

### N1/C3 SpyCas9

MGSSPPKKKRKVGGSTS-[SpyCas9 Core Sequence]-

GGSSYPYDVDPDYALEGGGSPKKKRKVGGSPKKKRKVGGRGGSPPKKKRKVGGSHHHHHH

### N2/C0 SpyCas9

MGSSPPKKKRKVGGSTSPKKKRKVGGSS-[SpyCas9 Core Sequence]-

GGSSYPYDVDPDYALEGGGSHHHHHH

### N2/C1 SpyCas9

MGSSPPKKKRKVGGSTSPKKKRKVGGSS-[SpyCas9 Core Sequence]-

GGSSYPYDVDPDYALEGGGRGGSPPKKKRKVGGSHHHHHH

### N2/C2 SpyCas9

MGSSPPKKKRKVGGSTSPKKKRKVGGSS-[SpyCas9 Core Sequence]-

GGSSYPYDVDPDYALEGGSPKKKRKVGGRGGSPPKKKRKVGGSHHHHHH

### N3/C0 SpyCas9

MGSSPPKKKRKVGGSTSPKKKRKVGGSPKKKRKVGGSS-[SpyCas9 Core Sequence]-

GGSSYPYDVDPDYALEGGGSHHHHHH

### N3/C1 SpyCas9

MGSSPPKKKRKVGGSTSPKKKRKVGGSPKKKRKVGGSS-[SpyCas9 Core Sequence]-

GGSSYPYDVDPDYALEGGGRGGSPPKKKRKVGGSHHHHHH

## N4/C0 SpyCas9

MGSSPKKKRKVGGSTSPKKRKVGGSPKKRKVGGSPKKRKVGGSS-[SpyCas9 Core Sequence]-  
GGSYPYDVPDYALEGGGSHHHHHH